Comparison of Insect Kinin Analogs With *cis*-Peptide Bond Motif 4-Aminopyroglutamate Identifies Optimal Stereochemistry for Diuretic Activity

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ABSTRACT:

The insect kinins are present in a wide variety of insects and function as potent diuretic peptides, though they are subject to rapid degradation by internal peptidases. Insect kinin analogs incorporating stereochemical variants of (2S,4S)-4-aminopyroglutamate (APy), a cis*-peptide bond motif, demonstrate significant activity in a cricket diuretic assay. Insect kinin analogs containing (2R,4R)-APy, (2S,4R)-APy and (2S,4S)-APy are essentially equipotent on an insect diuretic assay, with EC_{50} values of about 10^{-7}M, whereas the (2R,4S)-APy analog is at least 10-fold more potent ($EC_{50} = 7 \times 10^{-9}$M). Conformational studies in aqueous solution indicate that the (2R,4S)-APy analog is considerably more flexible than the other three variants, which may explain its greater potency. The work identifies the optimal stereochemistry for the APy scaffold with which to design biostable, peptidomimetic analogs with the potential to disrupt critical insect kinin-regulated processes in insects.*

Keywords: 4-aminopyroglutamic acid; cis*-peptide bond; β-turn mimetic; constrained insect kinin analog*

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INTRODUCTION

The insect kinins share a highly conserved C-terminal pentapeptide sequence Phe-Xaa-Xbb-Trp-Gly-NH_2, where Xaa can be Tyr, His, Ser or Asn, and Xbb can be Ala but is generally Ser or Pro.[1] They have been isolated from a number of insects, including species of Dictyoptera, Lepidoptera, and Orthoptera. The first mem-

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bers of this insect neuropeptide family were isolated on the basis of their ability to stimulate contractions of the isolated cockroach hindgut,[2,3] but they are also potent diuretic peptides that stimulate the secretion of primary urine by Malpighian tubules, organs involved in the regulation of salt and water balance.[4] In the migratory locust (*Locusta migratoria*) the insect kinins and the corticotropin releasing factor (CRF)-related peptide, co-localized in locust neurosecretory cells, act synergistically to stimulate Malpighian tubule fluid secretion.[4,5] In the housefly, muscakinin has been implicated in the control of diuresis in response to hypovolemia[6] and elicits a four- to fivefold increase in *in vitro* fluid secretion of the Malpighian tubules, more than twice the response observed with the larger CRF-related Musca-DP.[4,5] In addition, insect kinins, and/or analogs, have been reported to inhibit weight gain by larvae of the tobacco budworm (*Heliothis virescens*) and corn earworm (*Helicoverpa zea*),[7,8] both serious agricultural pests.

Structurally, the insect kinins require an intact C-terminal pentapeptide sequence for full cockroach myotropic and cricket diuretic activity, which therefore represents the active core.[9] An Ala-replacement analog series of the insect kinin active core region confirms the importance of the Phe and Trp side chains, because these are the only two replacements which lead to complete loss of myotropic and diuretic activity.[10–12]

Because of decreased conformational freedom, active cyclic analogs are more useful for defining the receptor-bound conformation than are linear analogs. Analysis of the conformations adopted by the head-to-tail, cyclic insect kinin analog *cyclo*(Ala-Phe-Phe-Pro-Trp-Gly), in which distance and angle constraints obtained from aqueous NMR spectra were incorporated into molecular dynamics calculations, indicated the most prevalent conformation featured a cis-Pro in the third position of a type-VI β-turn over core residues 1–4, or Phe-Phe-**Pro**-Trp (see Figure 1).[10–14] To obtain more evidence that this conformation represented the active one, analogs containing either tetrazole or 4-aminopyroglutamte (APy) moieties (see Figure 1) that preferentially form the 1–4 type VI β-turn[15] were synthesized and found to retain significant activity in a cricket diuretic assay.[16,17] A combination of NMR and computer modeling studies indicated that both the tetrazole and APy analogs induced a 1–4, type VI β-turn in aqueous solution.[16,17] As in the head-to-tail cyclic insect kinin analog, molecular dynamics calculations of the tetrazole indicated that the critical Phe[1] and Trp[4] side chains readily form an aromatic–aromatic interaction, leading to a common aromatic surface that is postulated to be an important component of insect kinin receptor interaction.[10–12,16,17] Further confirmatory evidence was found through the biological evaluation of analogs that feature the presumed minimal requirements of a *cis*-peptide bond turn mimic and the two critical side chains of Phe[1] and Trp[4], capable of forming an aromatic surface. Simplified analogs built on an amino piperidone carboxylate scaffold based on this receptor-interaction model demonstrated weak but demonstrable significant diuretic activity in a cricket diuretic assay.[18]

Here we report on the synthesis and biological evaluation of insect kinin analogs containing three stereochemical variants of the (*2S, 4S*)-4-aminopyroglutamic acid (APy) component (see Figure 1), a mimic of the *cis*-peptide bond.[17–20] These include the (*2R,4R*), (*2R,4S*), and (*2S,4R*) forms of Ac-Arg-Phe-**APy**-Trp-Gly-NH_2. The biological activities and solution conformations of the APy stereochemical variants are compared with one another and with three previously reported stereochemical variants of analogs containing the tetrazole moiety, also a mimic of a *cis*-peptide bond. The work leads to the identification of the optimal APy stereochemistry to use as a scaffold to design biostable, peptidomimetic analogs useful as tools to insect neuroendocrinologists and with the potential to disrupt critical processes in pest insects regulated by the insect kinins.

MATERIALS AND METHODS

Chemistry

The peptidomimetic analogs, Ac-Arg-Phe-(*2S,4R*)-APy-Trp-Gly-NH_2, Ac-Arg-Phe-(*2R,4R*)-APy-Trp-Gly-NH_2 and Ac-Arg-Phe-(*2R,4S*)-APy-Trp-Gly-NH_2 were synthesized manually by the solid-phase method, using the Fmoc-strategy and starting from Rink Amide resin (Novabiochem, 0.47 m*M*/g). The Fmoc protecting group was removed using 20% piperidine in DMF. A fourfold excess of the respective Fmoc-amino acids was activated *in situ* using HBTU (1 eq)/HOBt (1 eq) in DCM and coupling reactions were base catalyzed with DIEA (1 eq). Amino acid side-chain-protecting groups were Pbf for Arg and Boc for Trp. The coupling of Fmoc-4-aminopyroglutamic acids (Fmoc-aPy-OH, Fmoc-apy-OH and Fmoc-Apy-OH; aPy = (*2S,4R*)-APy, apy = (*2R,4R*)-APy, Apy = (*2R,4S*)-APy) derivatives during synthesis was mediated by PyBOP (instead of HBTU) to minimize racemization in the presence of DIEA in a mixture of NMP and DCM (1:1 v/v). The synthesis of enantiomerically pure Fmoc-4-aminopyroglutamic acids has been described elsewhere.[17,20–22] The completeness of each coupling reaction during synthesis was monitored by the Kaiser test. A second coupling was performed when the test was found positive. Cleavage of the peptide from the resin with side-chain deprotection was performed by treatment with TFA:H_2O:TIS (95.0:2.5:2.5 v/v/v, 10 ml/g peptide-resin) for 1.5 h. The cleaved peptides were precipitated with 10–20 volumes of diethyl ether, filtered, washed successively with more ether and air-dried. The resulting crude peptides were extracted with water and lyophilized.

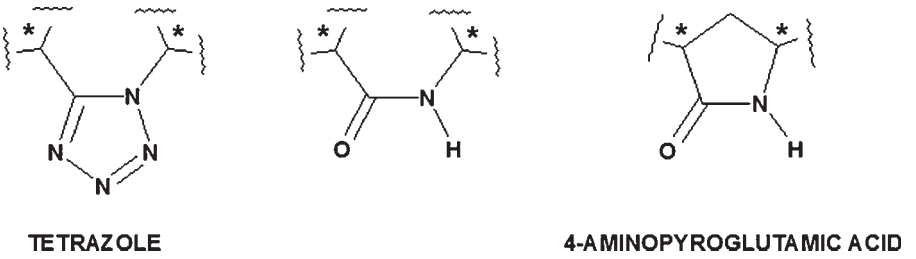

FIGURE 1 A comparison of the structures of the tetrazole (ψ[CN_4], left) and 4-aminopyroglutamic acid (APy; right) motifs, mimics of the *cis*-peptide bond (middle) and a type VI β-turn.

The peptidomimetic analogs were purified manually on a Waters C_{18} Sep Pak cartridge followed by a δ-Pak C_{18} reverse-phase column (8 × 100 mm^2, 15 μm particle size, 100 Å pore size) on a Waters 510 HPLC controlled with a Millennium 2010 chromatography manager system (Waters, Milford, MA) with detection at 214 nm at ambient temperature. Solvent A = 0.1% aqueous trifluoroacetic acid (TFA); Solvent B = 80% aqueous acetonitrile containing 0.1% TFA. Conditions: Initial solvent consisting of 20% B was followed by the Waters linear program to 100% B over 40 min; flow rate, 2 ml/min. δ-Pak C-18 retention times: Ac-Arg-Phe-(*2R,4R*)-APy-Trp-Gly-NH_2, t_R = 10.5 min; Ac-Arg-Phe-(*2R,4S*)-APy-Trp-Gly-NH_2, t_R = 6.5 min; Ac-Arg-Phe-(*2S,4R*)-APy-Trp-Gly-NH_2, t_R = 9.5 min. The analogs were further purified on a Waters Protein Pak I125 column (7.8 × 300 mm^2, Milligen, Milford, MA). Conditions: Flow rate: 2.0 ml/min; Solvent A = 95% acetonitrile made to 0.01% TFA; Solvent B = 50% aqueous acetonitrile made to 0.01% TFA; 100% A isocratic for 4 min, then a linear program to 100% B over 80 min. WatPro retention times: Ac-Arg-Phe-(*2R,4R*)-APy-Trp-Gly-NH_2, t_R = 8.75 min; Ac-Arg-Phe-(*2R,4S*)-APy-Trp-Gly-NH_2, t_R = 9.0 min; Ac-Arg-Phe-(*2S,4R*)-APy-Trp-Gly-NH_2, t_R = 7.75 min. These HPLC conditions have been described in detail elsewhere.[23] Amino acid analysis was carried out under previously reported conditions[23] and used to quantify the peptides and to confirm identity, leading to the following analysis: Ac-Arg-Phe-(*2R,4R*)-APy-Trp-Gly-NH_2; G[1.0], F[1.0], R[1.0]; Ac-Arg-Phe-(*2R,4S*)-APy-Trp-Gly-NH_2; G[1.2], F[1.0], R[1.0]; Ac-Arg-Phe-(*2S,4R*)-APy-Trp-Gly-NH_2; G[1.2], F[1.0], R[1.0]. The identities of the peptide analogs were confirmed via MALDI-MS on a Kratos Kompact Probe MALDI-MS machine (Kratos Analytical, Manchester, UK) with the presence of the following molecular ions (MH^+)[calc. MH^+ = 732.29]: Ac-Arg-Phe-(*2R,4R*)-APy-Trp-Gly-NH_2, 732.2 [MH^+]; Ac-Arg-Phe-(*2R,4S*)-APy-Trp-Gly-NH_2, 732.0 [MH^+]; Ac-Arg-Phe-(*2S,4R*)-APy-Trp-Gly-NH_2, 732.3 [MH^+].

NMR Spectroscopy

NMR spectra were acquired[14,16,17] on a Bruker ARX-500 500 MHz instrument using a 5-mm Z-gradient Bruker HCN probe. Samples of approximately 1 m*M* concentration were prepared in 0.5 ml 10% D_2O/H_2O with 0.1 m*M* DSS as internal standard and were placed in 5 mm × 7 mm Wilmad 535-PP sample tubes. All spectra were acquired in nonspinning mode. To determine if stable hydrogen bonds were present, temperature gradient experiments were performed consisting of a set of 1D spectra recorded between 5 and 45°C. WATERGATE solvent suppression was used, in an automated procedure developed in our laboratory utilizing an FTS Systems Air-Jet cooled nitrogen flow set at −20°C as cooling source. Based on N—H temperature shift coefficients, these experiments indicated that no permanent amide hydrogen bonds were present in the three analogs in the present study and all further experiments were performed at 22°C. Two-dimensional experiments were acquired using 512 increments of 1024 data points, with zero filling to produce 1024 data points in both dimensions, and data were transformed with shifted sine bell window function using the Bruker forward linear prediction correction algorithm. Peak assignments were primarily based on data derived from a TOCSY experiment acquired with a 57 ms mixing period and WATERGATE solvent suppression. Assignments were verified and distance constraints were obtained using data from a ROESY experiment with presaturation water suppression and a pulsed 9% duty cycle high-power spinlock[1] of 253 ms duration, acquired with water suppression through presaturation. For 2D spectroscopy a 1.8-s relaxation delay between experiments was used, incorporated in the ROESY experiment as the presaturation period. $^3J_{N-H\ H\text{-}\alpha}$ couplings were measured on the 1D spectrum taken at 22°C.

Molecular Modeling

Molecular modeling was performed in general by methods previously reported[14,16,17] using TRIPOS Sybyl 6.3 software running on SGI Indigo or O2 computers. The peptide was built using the Biopolymers subprogram with the turn promoting analog being prepared by modification of a proline residue. Based on data from the ROESY experiment described above, the following number of nontrivial distance constraints were included in the molecular modeling calculations; 13 for (*2S,4S*)-Apy[17], 15 for (*2R,4R*)-APy, 18 for (*2S,4R*)-APy, and 0 for (*2R,4S*)-APy. No correlations were noted bridging the residues on opposite sides of the APy components in any of the stereochemcial variants. ROESY peak intensities were approximated by counting contours. Geminal hydrogen ROESY intensities were used for reference as a strong correlation. Based on these intensities, distance ranges of 1.8–2.7 Å, 1.8–3.3 Å, and 1.8–5 Å were assigned to strong, medium, and weak correlations, respectively. All simulations were carried out *in vacuo*, with dielectric function in distance mode. A penalty of 200 kcal/(mol $Å^2$) was assessed for ROESY range violations. Nonbonded interactions beyond 8 Å were disregarded. Distance geometry was used to generate four unique starting conformations and 200 cycles of simulated annealing were performed on each set. Annealing consisted of heating to 1000 K for 100 ps and then exponential cooling to 200 K over 2000 ps. Annealed conformers were then energy minimized. The Tripos force field was used as Wiener values are not available for the

Table I Diuretic Activity of Peptidomimetic Insect Kinin Analogs in the Cricket *Acheta domesticus*

Peptidomimetic Analog	Stimulation of Malpighian Tubule Fluid Secretion, EC_{50} ($10^{-8}M$)	% Maximal Response	95% CI
Ac-Arg-Phe-(***2S,4S***)-**APy**-Trp-Gly-NH_2	14	93 ± 10	13.6–14.9
Ac-Arg-Phe-(***2R,4R***)-**APy**-Trp-Gly-NH_2	7	83 ± 6	3.2–17.2
Ac-Arg-Phe-(***2R,4S***)-**APy**-Trp-Gly-NH_2	0.7	93 ± 6	0.37–1.51
Ac-Arg-Phe-(***2S,4R***)-**APy**-Trp-Gly-NH_2	12	96 ± 1	11.3–13.6
Phe-**Phe**-[ψ**CN$_4$**]-**Ala**-Trp-Gly-NH_2	34	100[16]	
Phe-**D-Phe**-[ψ**CN$_4$**]-**D-Ala**-Trp-Gly-NH_2	58	100[24]	
Phe-**Phe**-[ψ**CN$_4$**]-**D-Ala**-Trp-Gly-NH_2		(43)[a16]	

[a] Partial antagonist. Natural insect kinin diuretic response is limited to 50% of maximal.

APy component. Kollman charges were used for normal amino acid components with Gasteiger–Huckel charges for the APy atoms. The three lowest energy conformers from each annealing experiment were compared. Two sets of the four were higher in energy and differed in conformation from the other three which were similar. The lowest energy form from this set of six was used in further studies. In further experiments, NMR constraints were removed from the lowest energy conformer and dynamics at 200 K for 100,000 fs were performed. This experiment indicated that the area near the APy moiety was fairly rigid but conformational changes occurred more readily as the two termini were approached. Low energy forms for all four of the stereochemical variants of the APy analogs are shown in Figure 3.

Biology

The diuretic assay has been described in detail elsewhere.[4] In brief, Malpighian tubules were removed from adult female crickets 6–12 days old and were transferred to 5 μl drops of bathing solution having the following composition: NaCl, 82 m*M*/l; KCl, 27 m*M*/l; $CaCl_2$, 2 m*M*/l; $MgCl_2$, 8.5 m*M*/l; NaH_2PO_4, 4 m*M*/l; NaOH, 11 m*M*/l; glucose, 24 m*M*/l; proline, 10 m*M*/l; Hepes, 25 m*M*/l. The pH was adjusted to 7.2 with 1*M* NaOH. The dissected tubules and associated saline droplets are held under liquid paraffin. Urine escapes from a cut made close to the proximal end of the tubule and collects as a discrete droplet in the paraffin. Urine samples are collected at intervals and their volume determined from measurements of droplet diameter under a microscope. After a 40-min equilibration period, the rate of secretion was measured over two 40-min periods before and after the addition of peptide analogs. Diuretic activity is calculated as the increase in fluid secretion (Δ nl/mm/min) and is expressed as a percentage of the response to a supramaximal dose (10 n*M*) of achetakinin-I assayed on Malpighian tubules taken from the same insect.

RESULTS

The insect kinin analogs Ac-Arg-Phe-(*2R,4R*)-APy-Trp-Gly-NH_2 and Ac-Arg-Phe-(*2S,4R*)-APy-Trp-Gly-NH_2 were found to stimulate cricket Malpighian tubule fluid secretion at EC_{50}'s of 7 and 12 × $10^{-8}M$; values which are not statistically different from the activity (EC_{50} = 14 × $10^{-8}M$) of the parent (*2S,4S*)-APy analog[17] (Table I). However, the diuretic activity of the (*2R,4S*)-APy stereochemical variant was found to be about 10-fold greater, with an EC_{50} of 0.7 × $10^{-8}M$. All of the APy analogs approach the maximal diuretic response of the native insect kinins (Figure 2). The diuretic activity of the three previously reported stereochemical variants of the insect kinin analogs containing the related tetrazole moiety (see Figure 1) are also placed in Table I to provide a comparison with the activities of the four APy stereochemical var-

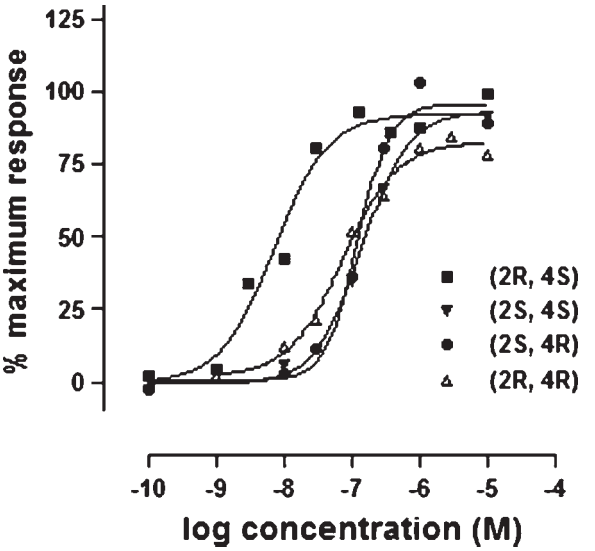

FIGURE 2 A comparison of the dose-response curves for the four stereochemical variants of the APy insect kinin analogs Arg-Phe-(*2S,4S*)-APy-Trp-Gly-NH_2, Ac-Arg-Phe-(*2R,4S*)-APy-Trp-Gly-NH_2, Ac-Arg-Phe-(*2S,4R*)-APy-Trp-Gly-NH_2, and Ac-Arg-Phe-(*2S,4R*)-APy-Trp-Gly-NH_2 in stimulation of Malpighian tubule fluid secretion in the cricket, *Acheta domesticus*. The (*2R,4S*)-APy analog is about 10-fold more potent than the other three.

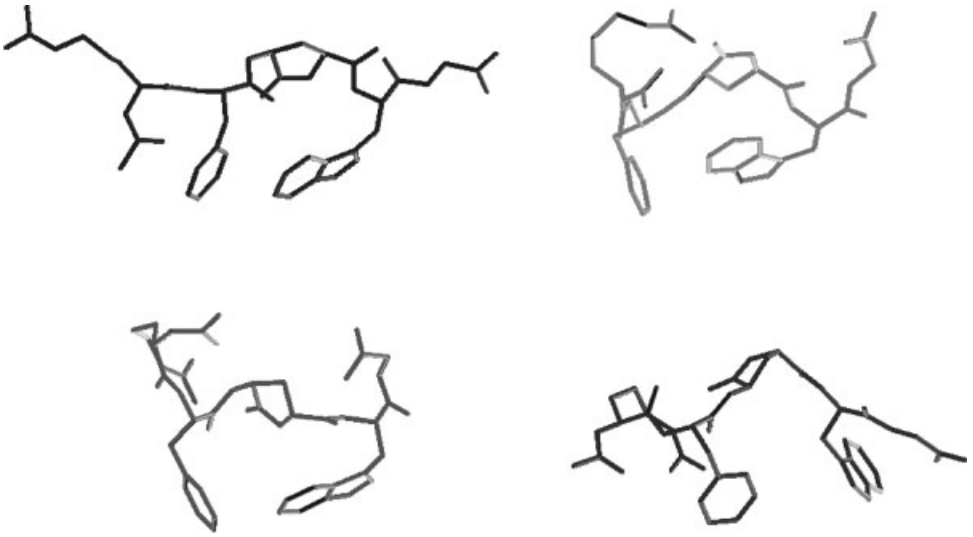

FIGURE 3 A comparison of low-energy conformations of insect kinin APy analogs Ac-Arg-Phe-(*2S,4S*)-APy-Trp-Gly-NH_2 (upper left), Ac-Arg-Phe-(*2R,4R*)-APy-Trp-Gly-NH_2 (upper right), Ac-Arg-Phe-(*2R,4S*)-APy-Trp-Gly-NH_2 (lower left), and Ac-Arg-Phe-(*2S,4R*)-APy-Trp-Gly-NH_2 (lower right); All four analogs demonstrate a preference for an open 1–4 β-turn, and the Phe-Trp side chains in all analogs readily form an aromatic-aromatic interaction (at underside of each analog), leading to a common aromatic surface that is postulated to be an important component of insect kinin receptor interaction.[9,10,14,16]

iants. The tetrazole-containing insect kinin analogs Phe-**Phe-[ψCN$_4$]-Ala**-Trp-Gly-NH_2[16] and Phe-**D-Phe-[ψCN$_4$]-D-Ala**-Trp-Gly-NH_2[24] were found to stimulate cricket Malpighian tubule fluid secretion at EC_{50}'s of 34 and 58 $\times$ $10^{-8}M$, respectively, whereas analog Phe-**Phe-[ψCN$_4$]-D-Ala**-Trp-Gly-NH_2[16] was found to partially antagonize the diuretic activity of the native insect kinins at an EC_{50} of 43 $\times$ $10^{-8}M$. The APy analogs as a group demonstrate greater potency than the tetrazole agonists; although the L,D-tetrazole analog is unique in that it can antagonize the diuretic activity of the native insect kinins.

The conformation in solution of the APy insect kinin analogs were investigated using a combination of NMR spectroscopic data and molecular modeling. The low-energy conformers exhibited an open turn structure with some flexibility; and the greatest flexibility was observed in the (*2R,4S*) stereochemical variant. While 13–18 nontrivial distance constraints were identified in aqueous solution NMR experiments on (*2S,4S*)-APy, (*2R,4R*)-APy, and (*2S,4R*)-APy variants, none were found for the (*2R,4S*)-APy analog. The models indicated fluxional conformations, with only intermittent hydrogen bond formation, although the general turn shape was maintained. An aromatic–aromatic interaction between the side chains of Phe and Trp is observed in each of the analogs which brings the two aromatic rings into close proximity, as observed in the (L,L)-tetrazole analog, the (*2S,4S*)-APy analog, other constrained analogs and native forms.[9,10,14,16,17,24] Low energy conformations of the four stereochemical variant APy, insect kinin analogs featuring an aromatic surface comprising an aromatic–aromatic interaction between the Phe[1] and Trp[4] aromatic side chains are depicted in Figure 3.

DISCUSSION

Previous studies have indicated that the restricted-conformation, tetrazole (see Figure 1) insect kinin analog Phe-Phe-ψ[CN_4]-Ala-Trp-Gly-NH_2 (L,L) and the 4-aminopyroglutamic acid (APy) (see Figure 1) analog Ac-Arg-Phe-(*2S,4S*)-APy-Trp-Gly-NH_2 (analogous to an L,L-stereochemical configuration) demonstrate significant activity in a cricket diuretic assay.[16,17] The tetrazole is a mimic of a *cis*-peptide bond type VI β-turn,[15–17,24–26] and using computer modeling data, the APy moiety (see Figure 1) has also been proposed by Paul et al. to mimic this turn.[19] The data from these two mimetic analogs therefore provided strong evidence for the active conformation of the insect kinins in the cricket diuretic assay. The (*2S,4S*)-APy insect kinin analog demonstrated an EC_{50} of 14 $\times$ $10^{-8}M$ and a 93% maximal response[17] (Table I), 2.5-fold more active than the **(L,L)-tetrazole** analog. The maximal response was not significantly different from that of the tetrazole analog.

The focus of this article is on the synthesis, biological evaluation, and solution conformation of the remaining three stereochemical variants of the APy, insect kinin analog: (*2R,4R*), (*2S,4R*) and (*2R,4S*). In the cricket diuretic bioassay, the (*2R,4R*)- and (*2S,4R*)-APy analogs demonstrated EC_{50} values (7 and 12 $\times$ $10^{-8}M$, respectively) that were not statistically different from the parent (*2S,4S*)-APy analog, whereas the (*2R,4S*)-APy analog proved to be about 10-fold more potent ($EC_{50} = 0.7 \times 10^{-8}M$) (Table I). A comparison with the analogous stereochemical variants of the tetrazole analogs indicate that, like the (*2S,4S*)- and (*2R,4R*)-APy analogs, the (L,L)- and (D,D)-**tetrazole** analogs are not statistically different from one another. The two tetrazole analogs are about threefold less potent than their APy counterparts. However, the relatively potent activity of the (*2R,4S*)-APy analog stands in stark contrast with the analogous (L,D)-**tetrazole** analog which demonstrates partial antagonism of the native insect kinins in the cricket diuretic bioassay. A comparison of the tetrazole counterpart of the (*2S,4R*)-APy variant cannot be made, as the tetrazole analog Phe-**Phe**-(D,L)-ψ**[CN$_4$]-Ala**-Trp-Gly-NH_2 has not yet been reported in the literature.

Molecular dynamics calculations incorporating constraints obtained from ROESY NMR experiments indicate that the low-energy solution conformations of the (*2R,4R*)- and (*2S,4R*)-APy analogs can readily approach an open 1–4 turn, featuring an aromatic–aromatic interaction between the Phe^1 and Trp^4 side chains as had been previously observed in other conformationally-constrained insect kinin analogs, including *cyclo*[AFFPWG] and analogs containing the tetrazole moiety.[10,14,16] This same conformation was reported for the parent (*2S,4S*)-APy analog.[17] In contrast, although more structured than the parent insect kinins, the (*2R,4S*)-APy analog proved to be considerably more flexible, with no major nontrivial NOE interactions observed. Nevertheless, molecular dynamics calculations also led to low-energy conformers that approach an open 1–4 turn and an aromatic surface comprising an aromatic–aromatic interaction between the Phe^1 and Trp^4 aromatic side chains. Figure 3 further illustrates this point with a comparison of low-energy conformations of all four stereochemical variants of the APy insect kinin analogs. The fact that all four stereochemical variant APy analogs demonstrate significant diuretic activity is likely due to the ability of each to adopt the aforementioned aromatic surface. Clearly, the three more rigid APy analogs (*2S,4S*), (*2R,4R*), and (*2S,4R*) each feature a different stereochemistry at the APy moiety; and yet the diuretic activity is not significantly different. Therefore, the 10-fold greater potency of the (*2R,4S*)-APy analog over the other three variants may well be a function of its flexibility, which would impart greater freedom to adopt a better fit with the receptor site.

The insect kinins are not biostable and thus are subject to rapid degradation by peptidases in the haemolymph (blood), tissues and gut of pest insects. The primary site of tissue-bound peptidase attack has been reported to be the amide bond between the Pro-Trp residues in the C-terminal pentapeptide core region.[8] The APy moiety precedes the Trp residue and would be expected to protect the adjacent amide bond from hydrolysis by peptidases. This work identifies the (*2R,4S*) stereochemical variant of the APy component as the best APy scaffold for the design of new, biostable, bioavailable agonist and/or antagonist analogs of this important class of insect neuropeptides. Future research efforts will focus on biostable, peptidomimetic analogs that can potentially disrupt the digestive and diuretic processes regulated by the insect kinins, with the potential to provide new candidates for tools for insect neuroendocrinologists and future pest insect control agents.

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